

Yeast electroporation

10×TE buffer (pH 7.5): 100 mM Tris-HCl, 10 mM EDTA

10× LiAc: 1 M LiAc, pH 7.5 (adjusted using HAc). Filter-sterilize or autoclave for 20 min.

1 M DTT: stored at -20°C

Day 1

Pick up single colonies on plates to 5 ml YPD medium. Culture at 30°C for 12-16 h.

Day 2

1) Inoculate into 50 ml YPD medium in flask. Culture at 30°C for 6-9 h.

<<<<<Put sterilized water on ice >>>>>

2) When OD is 0.5-1.2, transfer cell culture into 50 ml cap tube (sterilized).

<<<<< From here cells should be always on ice >>>>>

3) Collect cells by centrifugation (1100 g, 4°C, 5 min). Decant supernatant.

4) Re-suspend cells with 20 ml of sterilized H₂O (ice-cold). Mix with pipetman. Centrifuge and decant supernatant.

5) Treat cells with 20 ml of 0.1 M LiAc (16 ml 1 M sorbitol plus 2 ml 10×TE buffer plus 2 ml 1 M LiAc) at 30°C for 30 min. Add 0.2 ml 1 M DTT and keep cells at 30°C for 15 min. Centrifuge and decant supernatant.

<<<<<Put 1 M sorbitol on ice >>>>>

6) Wash cells twice with 20 ml of 1 M sorbitol (ice-cold). Centrifuge and decant supernatant.

7) Re-suspend cells with 100-200 µl of sterilized ice-cold 1 M sorbitol (final OD=100-200).

8) Take 50 µl of suspended cells into a new 1.5 ml tube on ice.

9) Add 5 µl fragment DNA (> 200 ng/µl). Mix with pipetman and keep on ice for 15 min. Transfer all to a sterilized cuvette (green cap). Add 1 ml of cold 1 M sorbitol to new labeled tubes (used later).

10) Set the cuvette in the holder of Micro Pulser Electroporator. Chose “Manual”, and set voltage at 1.5 kV. Push the pulse button. Read “Time / ms”, if it is between 4.0-6.0, this process is successful.

11) Add 1 ml of cold 1 M sorbitol, immediately after the pulse. Mix well by pipetting up and down. (After this, it is OK to be at RT.) Transfer all to the sorbitol tube ASAP.

12) Incubate at 30°C for 1-3 h. Centrifuge (3000 g, 1 min) to ~150 µl and then spread cells on selection plates. Make a negative control plate. Place the plates at 30°C air incubator.

YPD: yeast extract 10 g/l, peptone from meat 20 g/l, glucose 20 g/l.

SC-URA: YNB without aa 6.9 g/l, glucose 20 g/l, URA⁻ drop-out 0.77 g/l.

Delft: (NH₄)₂SO₄ 7.5 g/l, KH₂PO₄ 14.4 g/l, MgSO₄·7H₂O 0.5 g/l, metal solution 1 ml/l, vitamin 1 ml/l.